VARIATION IN GERMINATION AND GROWTH AMONG POPULATIONS OF AN INVASIVE PLANT, *ALLIARIA PETIOLATA* (M. BIEB) CAVARA AND GRANDE

A thesis presented to the faculty of the Graduate School of

Western Carolina University in partial fulfillment of the

requirements for the degree of Master of Science in Biology.

By

Carol Ann Petricevic

Director: Dr. Beverly Collins

Associate Professor of Biology

Biology Department

Committee Members: Laura DeWald, Biology

Jonathan Horton, Biology UNCA

Spring 2014

ACKNOWLEDGEMENTS

I thank my advisor, Dr. Beverly Collins, for her patience and encouragement for this thesis that proceeded on near geologic time-scale. Credit also goes to my committee members, Dr. Laura DeWald and Dr. Jonathan Horton for the further insights and challenging questions. I would also like to thank Dr. Tom Martin for all of his statistics help because otherwise it would have seemed like a foreign language. Thanks to all of the Biology Department that helped build up the mental library of ecological concepts over the years.

I am grateful to the people who have helped in the field and in the lab over the years. Hillrie Quinn, WCU Biology Department's Patton Center and Beverly Collins all provided land for my field plots. Thanks to the people at the state and national parks who helped me get permits for collection, and granted me free camping; I know permits can be a lot of paperwork, even if it's an invasive plant. All of my field workers were hard working, even when it was cold and wintery: Keith Hoffman, Karen Vaughn, Steve Whitehorn, and Jenna Kelly-Jones. Help in the lab was provided by: Chris Hart, Jennifer Torgerson, Lee Roddick, Liz Kessler, Kelly Grantham, and a whole group of UUs who counted numerous small seeds to get it done quickly. I would especially like to thank Lisa Coulson because of her encouragement and especially meticulous abilities in the lab; I would write her any number of recommendations as a lab assistant despite the fact that an MFA and conceptual art installations don't provide skills usually associated with biology. Bent Creek Experimental Forest was generous enough to provide me with lab space and assistance in Asheville, allowing me to spend less time driving! Keep on improving the data retrieval process, NOAA Climate Data Center, because it has definitely improved over the years, and thanks to the state climate office for assistance as well. My roommate/editors have been helpful and understanding of dirty dishes.

Graduate student sanity coaching was provided by Karrie Joseph, Tracy Myhalyk, and my dad, Mark Petricevic. Thanks to Elizabeth Mount and Beth Molman for edits. Patty Alman helped quite a bit with formatting and support in keeping my head together at the end. Thanks for the NPS for giving me the opportunity to work while I was in grad school, and for providing me with funny hats. Both of my parents, Mark and Judy Petricevic have been as lovingly supportive as good parents should be, even if my dad doesn't understand being interested in my side of biology. My grandfather, Ed Fassbender, has been great encouragement along the way, and I wish Grandma was still here to see the finished project. If I ever win the lottery, I will buy the school a new greenhouse.

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ABSTRACT

VARIATION IN GERMINATION AND GROWTH AMONG POPULATIONS OF AN INVASIVE PLANT, *ALLIARIA PETIOLATA* (M. BIEB) CAVARA AND GRANDE

Carol Ann Petricevic

Western Carolina University (April 2014)

Director: Dr. Beverly Collins

There are many different reasons why a non-native plant species might become invasive in a novel habitat. Some studies have focused on trying to determine the genetic structure of an invasive species. Other studies have investigated whether they are either more phenotypically plastic and thus able to utilize many different habitats, or it they have adapted to the new habitats before and during their range expansion. The invasive biennial *Alliaria petiolata* (M. Bieb) Cavara and Grande has been shown to be both phenotypically plastic and adapted to its introduced range. In this this study I investigated differences in survival, growth and reproduction and for multiple source populations of *A. petiolata* grown together in novel environments not experienced by any of the source populations. I conducted a common garden study as well as a cold-stratification germination study, utilizing seeds collected from source populations located along the species' invasive range. For the common garden experiment, seeds were collected in North Carolina, Tennessee, Virginia, Maryland, Pennsylvania and New York and placed in two garden plots at two different elevations, Franklin, NC (elevation 670m) and Highlands, NC (1190m). The cold stratification experiment used seeds from Asheville, North Carolina; Cleveland, Ohio; and Bronx, Cold Spring and Ossining, NY. These seeds were placed in moist soil and cold-stratified at 3°C for a short (66 days), medium (80 days) and long (100 days) cold stratification season.

The results of the common gardens study showed differences in responses among populations in numerous traits: seed and silique production, silique abortion, survival, number of stalks and biomass. When means were sorted from north to south with a calculated equivalent bioclimate using Hopkins Bioclimatic Law, adaptation to climate was potentially observed with a greater number of stalks in the southern equivalent bioclimate in the low elevation gardens.

The seeds from two northern populations of Cleveland, Ohio and New York ("H") showed the highest germination for the short stratification season of 1584 chilling hours, with both over 20% germination. The southern-most population in Asheville, North Carolina had the highest germination (86%) for the medium stratification season of 1920 chilling hours. As previous studies have also shown, germination was nearly complete with 2400 hours of cold-stratification.

There may be adaptation to climate among the populations studied for number of stalks, but no other traits show any patterns with regards to climate. A few traits such as seed production, number of siliques and survival, showed similar means among the northern populations, and may indicate genetic relationships among populations along the southward invasion route of *A. petiolata's* range. This study supports the conclusion that *A. petiolata* is a habitat generalist, but that there is some variation in growth, reproduction and survival.

INTRODUCTION

Alliaria petiolata (M. Bieb) Cavara and Grande, or garlic mustard, is a species invasive to North America, that has spread to most of its predicted range in forested eastern North America (Welk et al. 2002). *Alliaria petiolata* was introduced in Long Island, New York in the mid-1800s, initially spread westward, then southward (Nuzzo 1993). In North America, *A. petiolata* ranges from southeastern Canada, east to the plains states and south into Virginia, but does not commonly grow south or east of the Appalachian mountains in the Piedmont or Coastal Plain of NC, SC, or GA. Welk et al. (2002) hypothesize it is unlikely the range will extend to the Coastal Plain due to climatic constraints. However, *A. petiolata* did have a reproducing population at Kennesaw Mountain in suburban Atlanta (personal observation) and EDDMAPS indicates populations near Durham, and Fayetteville, NC (EDDMAPS 2014), suggesting expansion to the Piedmont may be possible if the plant is introduced to cooler, protected micro-climates that are similar to its habitat distribution in its native range (Welk et al. 2002).

Spread of Invasive Species and Differences Among Populations

A common question in invasive ecological studies is whether invasive plants have to adapt to local conditions before becoming invasive, or if they are intrinsically phenotypically plastic (Parker et al. 2003; Richards et al. 2006). Phenotypic plasticity is the ability of organisms to show different morphologies, behavior, or physiology that varies due to environmental factors (Richards et al. 2006) and is considered important for initial introduction success (Baker 1974, Kowarik 1995; Sakai et al. 2001; Crooks 2005; Theoharides and Dukes 2008). In essence, plasticity is flexibility in requirements that allows the plant to live in varying conditions (Richards et al. 2006).

Some species may be phenotypic plastic in general, while others may evolve plasticity after introduction (Richards et al. 2006). Some traits may be considered to have “adaptive” phenotypic plasticity, while others may merely be environmentally plastic (Ghalambor et al. 2007; van Kleunen et al. 2011), which means that the trait varies due to the environment but does not confer a fitness advantage. It has been assumed that selection that might act on a plastic phenotype will not allow for evolution (Ghalambor et al. 2007), as the plasticity may provide a buffer against selection on the genotype. Others argue that phenotypic plasticity can create conditions where the genes follow the environment and may eventually no longer need the environmental stimulus to provide that trait and may instead facilitate adaptive evolution, alternatively described as “genes as followers” (Ghalambor et al. 2007). An alternative way of describing phenotypic plasticity is as the “jack of all trades,” where the plasticity of the invader allows the species to maintain fitness in many different environments, “master-of some,” where the plasticity of the invader allows the species to be highly fit in its favorable environments, or “jack-and-master,” which combines these traits (Parker et al 2003; Richards et al. 2006).

Alliaria petiolata’s invasion history and large range in North America raises the question of whether it has adapted to new habitats during range expansion or, alternatively, is an intrinsically plastic species. *Alliaria petiolata* underwent a lag phase, remaining within a small section of Long Island, New York, in the first 20 years after introduction, but became widespread in the following 20 years, spreading exponentially (Nuzzo 1993). Lag times have been suggested as the time needed to adapt to local conditions (Crooks 2005). Genetic studies have shown that there are some novel North American alleles in *A. petiolata* (Durka et al. 2005), and previous common garden studies show introduced populations have reduced competitive ability, loss of resistance to specialist herbivores (Bossdorf et al. 2004a & b), and loss of allelopathic potential

compared to their native counterparts (Cipollini 2002; Lankau et al 2009; Hillstrom and Cipollini 2011; Cipollini and Liurance 2012). Other studies have shown phenotypic plasticity in flowering phenology (Byers and Quinn 1998), defensive chemical production (Cipollini 2002), biomass allocation, number of leaves, seed production (Meekins and McCarthy 2000) and ovule and fruit location (Susko and Lovett-Doust 1998), which are affected by resource availability.

The objective of this research was to determine if *Alliaria petiolata* plants grown from seeds taken from populations within the invasive range show variation in traits, such as seed production or vegetative mass, with respect to different environments and climates found at two elevations in the Southern Appalachians (length of the growing and dormant seasons) that could affect the species' ability to invade into more habitats of the Southern Appalachians and beyond. Common garden experiments have a long history of facilitating investigations of the differences among populations or genotypes when grown together (Clausen et al. 1940). Common garden experiments are particularly useful when investigating environmental and genetic reasons for the success of invasive plants (Moloney et al. 2009). I conducted common garden experiments to test the following hypotheses:

I. *Alliaria petiolata* populations from across the invasive range grown under different dormant and growing season lengths in a common garden will differ in over-winter survival, second year survival, plant mass, or fitness (number of seeds set and mass of seeds), but show no relation to equivalent bioclimate.

II. Alternatively, populations will vary by aligning with their equivalent bioclimate in responses in survival, mass, and fitness, in environments that more closely match the environment from which the seeds were taken, which may indicate local genetic adaptation.

Cold Stratification Requirements

Cold stratification is necessary in some plants to break dormancy. *Alliaria petiolata* requires cold stratification to germinate, with studies showing reliable germination occurring after 2400 chilling hours of cold stratification between the temperature of-1°C and 6°C, with 2160 chilling hours being the minimum for significant germination success (Raghu and Post 2008).

Alliaria petiolata may show adaptation to the climate where it occurs in regards to cold stratification requirements, with colder climates requiring a longer cold stratification period to induce germination. It is possible that *A. petiolata's* range is constrained by its cold stratification requirements that may not be met in Piedmont or Coastal Plain of the Southeast, where it is not highly established. Alternately, *Alliaria petiolata* may not show differences among the germination requirements of different populations. Other species have shown specific niche adaptation in dormancy and range. For example, *Osmorhiza depauperata* (Coult. & Rose) Fern. has shown divergent evolution in warm stratification utilization, with populations from warmer climates benefitting from increased germination if seeds are warm stratified after cold stratification (Walck and Hidayati 2004). *Lamium spp.* L have adapted to warm stratification requirements (Karlsson and Milberg 2008). Range constriction due to stratification occurs in *Vitis vinifera* L. (Martino et al. 2012).

The objective of the germination study was to determine if different populations of *A. petiolata* have different stratification requirements, as differences among populations may indicate that *A. petiolata* has adapted locally along the invasion route. It is possible that *A. petiolata* adapted to different cold stratification time-lengths for different areas within the invaded North American zones, such as more northern populations requiring a longer cold stratification period to break dormancy. I examined germination rates with different moist stratification times, short (1584 chill hours), medium (1940 chill hours)

and long (2400 chill hours), with seeds from 5 populations. I tested the following hypotheses:

I. Germination percentages will differ among populations in the three cold stratification treatments and population response will correspond with the collection site climate, indicating possible local adaptation to site climate.

II. Alternatively, germination percentages will not differ among populations, or the populations will differ in germination rates for the three cold stratification treatments, but will not correspond with collection site climate.

BACKGROUND

Alliaria petiolata Biology

Alliaria petiolata, or garlic mustard, is a herbaceous biennial in the *Brassicaceae* family. Its native range extends from Greece and Italy north to Sweden and the United Kingdom and east to Russia, with small populations in Iraq, North Africa, Tajikistan and Nepal (Welk et al. 2002). *Alliaria petiolata* was introduced to Long Island, New York in the mid-1800s for its edible and medicinal qualities (Grieve 1931). It has since been deemed an invasive species because of the exponential rate of spread and number of habitats it has invaded. Its introduced range in North America reaches north to Ontario, south to western North Carolina, and west to the Plains states. There are also some small, scattered populations in Oregon, Washington, British Columbia, Colorado, and Utah (Welk et al. 2002; Rodgers et al. 2008b).

In Europe, *A. petiolata* is found in moist humid areas along riversides and roadways and tends to occur on north-facing slopes in hotter, drier climates (Welk et al. 2002). In North America, it often is found on moist soils in edge forests, especially along roadways, trails, and other edge habitats (Welk et al. 2002). However, it is also shade tolerant and does not need disturbance to become established into an ecosystem, unlike most other invasive plants (Rodgers et al. 2008a).

In North America, *A. petiolata* germinates in the spring, starting in February in the warmest climates and as late as June in the northern most parts of its range (Cavers et al. 1979). *Alliaria petiolata* develops as a basal rosette during the first growing season, remains green over winter even under snow, bolts the next spring, and sets seed in June through August. When the plants are in temperatures above freezing and not covered in snow, they can continue to grow (Cavers et al. 1979). Dry stalks hold siliques that, when ripe, eject seeds explosively upon disturbance (Anderson et al. 1996). Early germination

and extended growing time before the canopy leafs out allows *A. petiolata* to have a competitive advantage over native spring ephemerals that have not yet emerged (Myers and Anderson 2003).

Alliaria petiolata averages 16.4 seeds per silique and 21.8 siliques per plant, making an average of 609 seeds per plant (Nuzzo 2000); large plants can produce over 2000 seeds (Cavers et al. 1979). Most plants produce 1-2 stalks, but they may have up to 12 stalks (Nuzzo 2000). There is high plant mortality (95%) through the first season, with continued mortality throughout winter. Survival to second-year adult was documented as 1% (Nuzzo 2000) or 2-4% (Cavers et al. 1979).

Alliaria petiolata is a competitive understory herb due to its allelopathy with chemicals that inhibit germination (Prati and Bossdorf 2004), reduce survival or growth of other plants (Vaughn and Berhow 1999), or hinder mycorrhizae, which many plants need for growth (Stinson et al. 2006; Wolfe et al. 2008). *Alliaria petiolata* has a pungent smell reminiscent of garlic as well as mustard greens and is well protected from herbivory by organic anions containing sulfur and glucose called glucosinolates that characterize much of the *Brassicaceae* family (Vaughn and Berhow 1999; Cipollini 2002).

Spread of Invasive Species and Differences Among Populations

Phenotypic plasticity is the ability of organisms to show non-genetic based morphology, behavior, or physiology that differs with environmental factors, allowing the plant to live in varying conditions (Richards et al. 2006). Plasticity is considered an important characteristic of weedy and invasive species because it allows a species to express high fitness in a broad range of environments (Richards et al. 2006). In *Polygonum cespitosum* Blume, a newly identified invasive plant in northeastern North America originating in Asia, plasticity has been shown in individual fitness, life-history, and functional traits. In a greenhouse study comparing introduced and native range

populations, the researchers found invasive populations of *P. cespitosum* would alter response with increasing allocation to root tissues, water use efficiency, and photosynthetic rate in drier, sunnier conditions. Both populations of plants grown in shady and moist understory-like situations responded similarly whether the seed source originated in a sunny and dry location or a moist understory condition (Matesanz et al. 2012). The researchers also found one native population was less plastic than introduced populations, though without more native seed sources they could not determine whether the enhanced plasticity originated before or after introduction to North America (Matesanz et al. 2012).

Local adaptation can confer competitive advantage, although it generally entails a lag-time before populations increase and ranges spread (Crooks 2005; Theoharides and Dukes 2008). Lag phases after introduction can be due to factors such as landscape fragmentation, limited dispersal, and time needed to adapt to local conditions, particularly if the population's genetic diversity is low, like many invasive species (Cousens and Mortimer 1995; Sakai et al. 2001). Low genetic diversity may require more time for mutations and gene combinations to develop before adaptation will occur (Sakai et al. 2001). Examples of adaptations which are heritable and differ among populations exist in introduced species. One example is the *Oncorhynchus tshawytscha* (Walbaum in Artedi) (Chinook salmon), which evolved different spawning times when introduced in New Zealand, suggesting rapid evolution (Quinn et al. 2000). Introduced *Prunella vulgaris* L. was shown to have adapted to closed-canopy conditions in a South American temperate rainforest (Godoy et al. 2011). In a *P. vulgaris* reciprocal transplant study between open site plants and shady rainforest plants, the plants from the forest grew taller and had greater specific leaf areas than the open site plants when grown in the shade and therefore were better shady site competitors (Godoy et al. 2011).

Some plants show both plasticity and local adaptation. In *Tamarix ramosissima* Ledeb., morphology and gas exchange were plastic under different growth chamber treatments, however Sexton et al. (2002) found local adaptation for root biomass in relation to climate, with northern ecotypes producing greater biomass. Adaptation in flowering timing was found in an Australian invasive clover, *Trifolium subterraneum* Katzn. & Morley (Cocks and Phillips 1979).

In a genetic study of *A. petiolata* involving 27 populations in Europe and 26 in North America, low heterozygosity and high inbreeding tolerance was noted in the introduced and native ranges. *Alliaria petiolata* often self-pollinates and highly selfing plants commonly have low heterozygosity and high inbreeding tolerance. While the genetic diversity in *A. petiolata* is low even in its native range there is less genetic variability in the introduced populations compared to the native populations (Durka et al. 2005). A recently released study indicated that *A. petiolata* is affected by founder effects, and that deleterious negative alleles can become fixed into an inbred population of *A. petiolata* and not selected out due to small genetic diversity (Mullarky et al. 2013).

After its introduction, *A. petiolata* rapidly spread after an initial lag time of 20 years with increasing rates of expansion, from an estimated 300 square kilometers to 6400 square kilometers per year (Nuzzo 1993). Sakai et al. (2001) suggested that introduced plants with low genetic diversity may need a lag time to become invasive, with rate of change in genetics proportional to the genetic variation present. *Alliaria petiolata* may have spread west first, then spread south (Nuzzo 1993; Lankau et al. 2009), with range jumps likely due to human-aided spread. The genetic study mentioned above also found some North American specific alleles in *A. petiolata* (Durka et al. 2005). The novel alleles are grouped from the oldest populations in the New York and New Jersey area and heading south, while the populations without the novel alleles are grouped in the Midwest (Durka et al. 2005). It is possible the species spread west first

with alleles similar to the originally introduced plants from Europe, then later, the oldest populations in the New York area evolved new alleles. These new alleles then spread with the southward expansion. The lag time may suggest some adaptation was required for initial spread, though later adaptation occurred as well (Nuzzo 1993).

Regionally specific alleles combined with information that introduced populations showed reduced competitive ability, allelopathic potential, and reduced resistance against specialist herbivores compared to plants from the native range strengthens the argument that some adaptation has occurred in introduced *A. petiolata* (Bossdorf et al. 2004 a & b). Introduced *A. petiolata* is less intra-specifically competitive when grown together with plants from its native range. The reason suggested for this is selection for stand fitness over individual fitness (Bossdorf et al. 2004a). The introduced plants were also defended less against specialist herbivores from the plant's native range (Bossdorf et al. 2004b). In some invasive species, selfing or vegetative reproduction can contribute to rapid local spread of phenotypically plastic or locally adapted genotypes (Daeler 1998, Durka 2005). Some invasive organisms have become more invasive through inbreeding (Tsutui 2000). For example, *Linepithema humile* (Mayr, 1868), an Argentine ant, was introduced to California as a small initial population that became inbred and grew into supercolonies that expanded and out-competed native ants (Tsutui 2000). This is unlike how the colonies form in its native range, which are much smaller.

Alliaria petiolata has been shown to be quite plastic, with plasticity demonstrated in reproductive characteristics such as ovule and fruit location due to resource limitation (Susko and Lovett-Doust 1998). Source populations responded plastically between floodplain and uplands in flowering phenology (Byers & Quinn 1998). Resource availability affects seed production (Nuzzo 2000), number of leaves, above-ground biomass, total biomass (Meekins and McCarthy 2000) and defensive chemical production (Cipollini 2002). Leaf chlorophyll production increases under low light

concentrations, but decreases in brighter light (Meekins and McCarthy 2000). In all of these instances, different source populations responded similarly to the treatments and in the environment. A study of native vs. introduced source populations in the common garden found variation in responses of traits to various treatments of plants grown from seed collected in Ohio, Pennsylvania, Germany, Netherlands and Sweden. Results showed variation in mean levels of trait responses among the populations for anti-herbivore, antioxidant and morphological traits. There were differences in the amount of plasticity among populations but not between continents (Hillstrom and Cipollini2011).

Cold Stratification of Alliaria petiolata

Moist cold stratification is needed for germination in a number of different species, including *A. petiolata*. There is some variation within species in dormancy breaking requirements. In a study looking at variation in dormancy breaking requirements in *Lamium spp.* L. (deadnettle), variation was found in the amount of warm stratification needed to germinate (Karlsson and Milberg 2008). Variation in germination strategies was shown in three closely related *Osmorhiza* species. Western North American *Osmorhiza depauperata* Phil., had deep complex morphophysiological dormancy (MPD). This means the seed requires its appropriate dormancy breaking treatment and the embryo in the seed is undeveloped and cannot grow if excised. The eastern North American species, *O. claytonii (*Michx.) C.B. Clarke and *O. longistylis* (Torr.) DC. had a different type of dormancy, nondeep complex MPD. This dormancy is similar to MPD, however, the embryo is developed to a level that, if it is removed from the seed it may develop normally (Walck et al. 2002; Baskin and Baskin 2004; Walck and Hidayati 2004). The study also determined warm stratification was not needed in *O. depauperata*, but germination of seeds from populations within the species improved after dormancy break if warm stratification occurred before the cold stratification period. This may indicate adaptation toward a seed requiring both warm and cold stratification

due to the different habitats in which they now grow (Walck and Hidayati 2004). Environmental effects altered germination rates in plants from dry versus moist sites, in *Polymnia canadensis* L., an eastern North American forest herb. However, a reciprocal transplant experiment showed no adaptation and that the germination differences were due to what the environment could provide seasonally (Bender et al. 2003). In a study of *Vitis vinifera* sub sylvestris (C.C. Gmel.) Hegi in Sardinia, climate change possibly effects seedling recruitment in lower elevation populations due to reduced germination from shortened cold stratification seasons, while seeds continued to germinate in the cooler, higher elevations. This means that climate may potentially define a species range (Walck et al 2011; Martino et al. 2012).

*Alliaria petiolata'*s dormancy is generally broken with cold stratification, but dormancy has been broken experimentally with scarification (mechanical or subjected to H_2SO_4) followed by application of gibberilic acid. This response means that *A. petiolata* does not show deep physiological dormancy (Sosnokie and Cardina 2009). More testing would be required to determine if *A. petiolata* exhibits non-deep or intermediate dormancy (Baskin and Baskin 2004). *Alliaria petiolata* seeds are water permeable before treatment, but are not gibberilic acid permeable until after scarification treatments (Sosnokie and Cardina 2009).

Alliaria petiolata showed near 100% germination after 2400 hours of cold stratification between the temperature of 1°C and 6°C, with 2160 hours being the minimum for a significant number of germinants (Raghu and Post 2008). In an early study of *A. petiolata,* Kinzel (1927) found that seeds need to be above freezing, but below 6°C for germination. Only one study from the native range in the former Czechoslovakia utilized multiple populations in a single germination study. This study found differences among populations, with one population having 18% germination at only 1440 hours of cold stratification, and another site having over 30% germination by

1920 hours. Two populations had no germination until after 2400 hours, including one that had only 2% germination and continued to 66% of total seeds by 2880 chill hours. These findings demonstrate some differences among populations with respect to germination, even within a small area inside the native range, but do not indicate adaptation to climate (Lhotská 1975). Baskin and Baskin (1992) found that *A. petiolata* has specific germination requirements; seeds do not require light but have low germination rates when placed on paper or sand instead of soil. No mechanism was offered in the study, but the author believes that it may be moisture sensitivity (C. Baskin, personal communication). A valuable future study would determine why *A. petiolata*, unlike most other seeds, requires soil and moist cold stratification to germinate (Baskin and Baskin 2001).

METHODS AND MATERIALS

Common Garden Experiment

The common garden experiment was conducted in two forest understory plots as the low elevation gardens in Franklin, NC (elevation 670 m) and two plots as the high elevation gardens in Highlands, NC (elevation 1500 m). The gardens were populated with plants grown from seeds collected along a south to north corridor from Asheville, NC up to the Hudson Valley region of New York. The plants were started in pots with potting soil (Fafard™4P) from seedlings in the Western Carolina University greenhouse and t placed in the field in late spring. Because the plant is a biennial, there were two times that measurements were made, once during the dormant winter season with measurements in the field, followed by a second round of measurements after the plants were removed from the field in April 2010 to finish their life-cycle in the greenhouse.

Alliaria petiolata seeds from the eastern range were collected from 14 populations, primarily along the I-81 corridor, in late July and early August, 2008 (Fig. 1, Table 1). Two collections of seeds, one from Armonk, New York and another from the Peaks of Otter in Virginia, were mailed to me, the rest I collected. The samples were gathered using a sweep net, which was passed over the siliques in the population and collected seeds from numerous plants. The seeds were placed in plastic bags and labeled and collection points were marked by GPS. The bags were stored in a climate-controlled office until being placed into cold stratification. In the lab, the seed collections were placed in a moist peat-lite soil mix (Fafard™ seed-starting mix: sphagnum moss and perlite) and were stored in a refrigerator at 3 °C, where they remained in cold stratification for 2400 hours to ensure germination (Raghu and Post 2008).

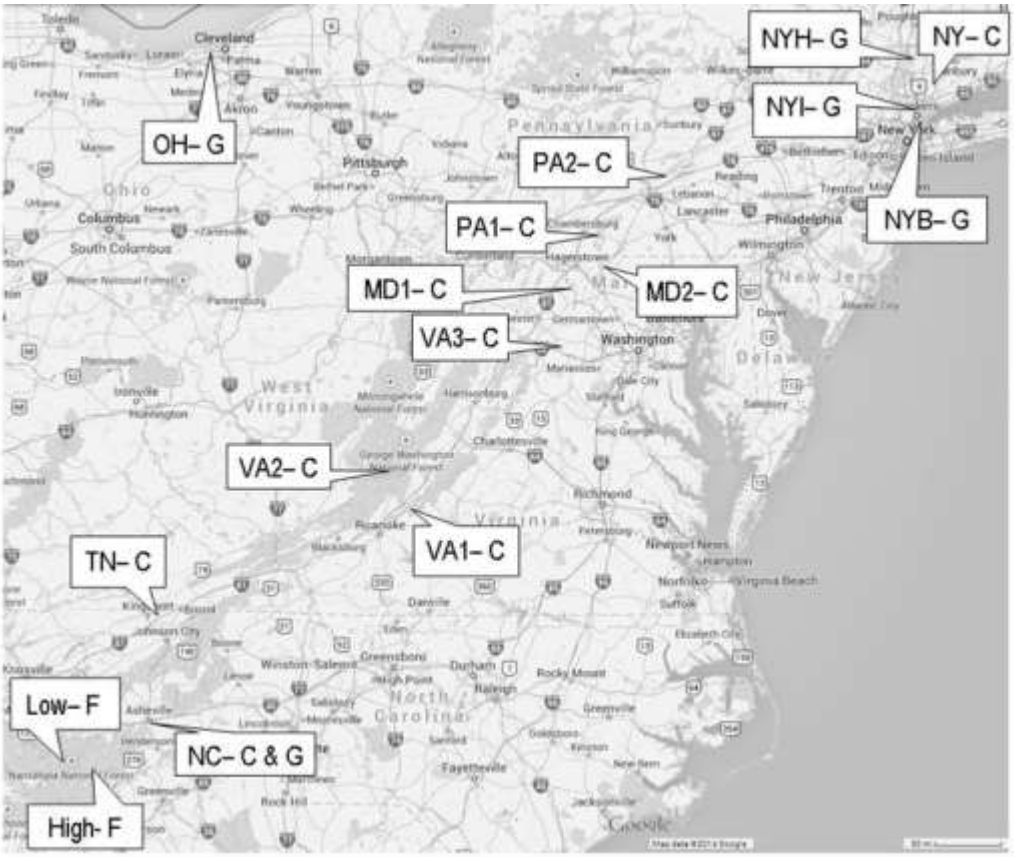

Figure 1. Field plots and seed collection locations shown for both the common garden and germination experiments. Field sites are indicated with an “F,” common garden study populations are indicated with a “C” and germination study populations are indicated with a “G.” Seeds from many plants in each population were collected together in a sweep net, and winnowed to remove siliques from seeds. Map generated on Google Maps (Google 2014).

For the common garden experiment, low and high elevation field sites were chosen to minimize differences other than elevation (e.g., in moisture, drainage, canopy conditions). Two sites, approximately 6.5 km apart, were chosen in Franklin, NC at the lower elevation (670m). These sites were dominated by common or exotic deciduous and evergreen trees, such as *Pinus strobus* L.*, Acer rubrum* L*, Paulownia tomentosa* (Thunb.) Steud*., Carya. spp.* Nutt.*,* and *Liriodendron tulipifera* L*,* and an herbaceous layer. Two higher elevation sites (1190m) approximately 60m apart were chosen in Highlands, NC. These sites were dominated by mixed high elevation forest species which included *Fagus grandifolia* Ehrh*., Betula alleghaniensis* Britton*, Quercus rubra* L.*, Pinus strobus* L.*,* and *Acer saccharum* Marsh. One of the higher elevation sites was bordered by planted *Abies fraseri* (Pursh) Poir*.* trees.

Seedlings for the low elevation gardens were started in the greenhouse in early March, 2009, in 12.7cm diameter peat pots and planted in the field on April 25th, 2009. The high elevation seedlings were started in the greenhouse in early April and planted in the field sites on May 17th, 2009. The start dates were staggered to mimic the differing germination times that would occur in different climates (Cavers et al. 1979; Nuzzo 2000). As shown in other research (Cavers et al. 1979; Nuzzo 2000), there was high seedling mortality in the greenhouse. Due to this high seedling mortality, I started the high elevation seeds in peat pellets and then transferred the seedlings to peat pots when moving to the field. This was done to lessen the risk of losing small, ungerminated seeds into the field, as the seeds were easier to monitor in peat pellets. The plants in the greenhouse were watered whenever the soil became dry, usually every two days. The plants were fertilized once with a MiracleGro™ solution (15mL of mix to 3.8L of water) during the month in the greenhouse and twice in the field later in the season, to ensure adequate soil nutrients in pot-bound plants.

At the end of the first greenhouse period, 15 individuals from each population group were placed in a randomized grid pattern to reduce garden placement factors. This design yielded 15 plants per population x ten populations yielding 150 plants per plot x two gardens at each elevation. The high level of seedling mortality meant that fewer than 30 plants per population were placed in the field in the low elevation gardens. The *A. petiolata* seedlings were planted in peat pots with Fafard™ 4P potting mix, so that the pots could be buried into the duff layer. Gardens were prepared in the field by clearing the herbaceous layer and pushing aside the duff layer, placing the pots into the grid, and then surrounding them with the duff and extra pine mulch after placement. The plants were watered equally when rain was not consistent to reduce mortality from drought; watering was not necessary during much of the growing season.

Two i-buttons (Maxim Integrated™) per plot were placed in doubled zipper storage bags to record temperatures, and at least one remained functional per garden plot throughout the experiment. Temperatures were recorded every two hours from December 2009 until plants were removed from the field and returned to the greenhouse, in March, 2010 for the low elevation plants and in April, 2010 for the high elevation plants (Fig. 3).

Plant traits were measured in the field in the winter of 2009 and at the end of the experiment when they were returned to the greenhouse. The winter measurements were carried out in December 2009 through March 2010, starting with the high elevation gardens until snow fell and made them inaccessible until March. The low gardens were measured in January when not covered in snow. Measurements made in the winter were survival, number of leaves per plant and number of leaves showing herbivory per total number of leaves. Herbivory index was measured as number of leaves showing herbivore damage over total number of leaves.

NY	NC			NY	VA2	MD1	NY	
PA1	MD2	VA3	NC	PA1	MD2	VA2	NC	PA1
TN	VA1	PA2	VA3	TN	VA1	MD2	VA3	TN
MD1	VA2			PA2	MD1	VA1	PA2	
	TN	VA1	VA3	TN			MD2	VA3
PA2	MD1	NY	VA1	PA2	MD1	TN	VA1	PA2
VA2	NC	PA1	NY	VA2	NC	MD1	NY	VA2
	MD2	VA3	PA1	MD2			NC	PA1
VA2	NC			NY	VA2	MD1	NY	
PA1	MD2	VA3	NC	PA1	MD2	VA2	NC	PA1
TN	VA1	PA2	VA3	TN	VA1	MD2	VA3	TN
MD1	NY			PA2	MD1	VA1	PA2	
	PA2	MD1	VA1	PA2			PA1	MD2
NY	VA2	NC	MD1	NY	VA2	VA3	TN	VA1
PA1	MD2	VA3	NC	PA1	MD2	PA2	MD1	NY
	TN	VA1	VA3	TN			VA2	NC
PA1	NC			PA2	MD1	VA1	PA2	
MD2	VA3	TN	NY	VA2	NC	MD1	NY	VA2
VA1	PA2	MD1	PA1	MD2	VA3	NC	PA1	MD2
VA2	NY			TN	VA1	VA3	TN	

Figure 2. Garden plot design for the *Alliaria petiolata* field gardens with15 plants per population. Each code represents a source population. The bolder outline represents a block, while the finer lines represent the space for a single plant. Real world placement required some variation in exact shape due to trees, roots, and other barriers. In the low gardens, there were not enough plants to perfectly use this plan.

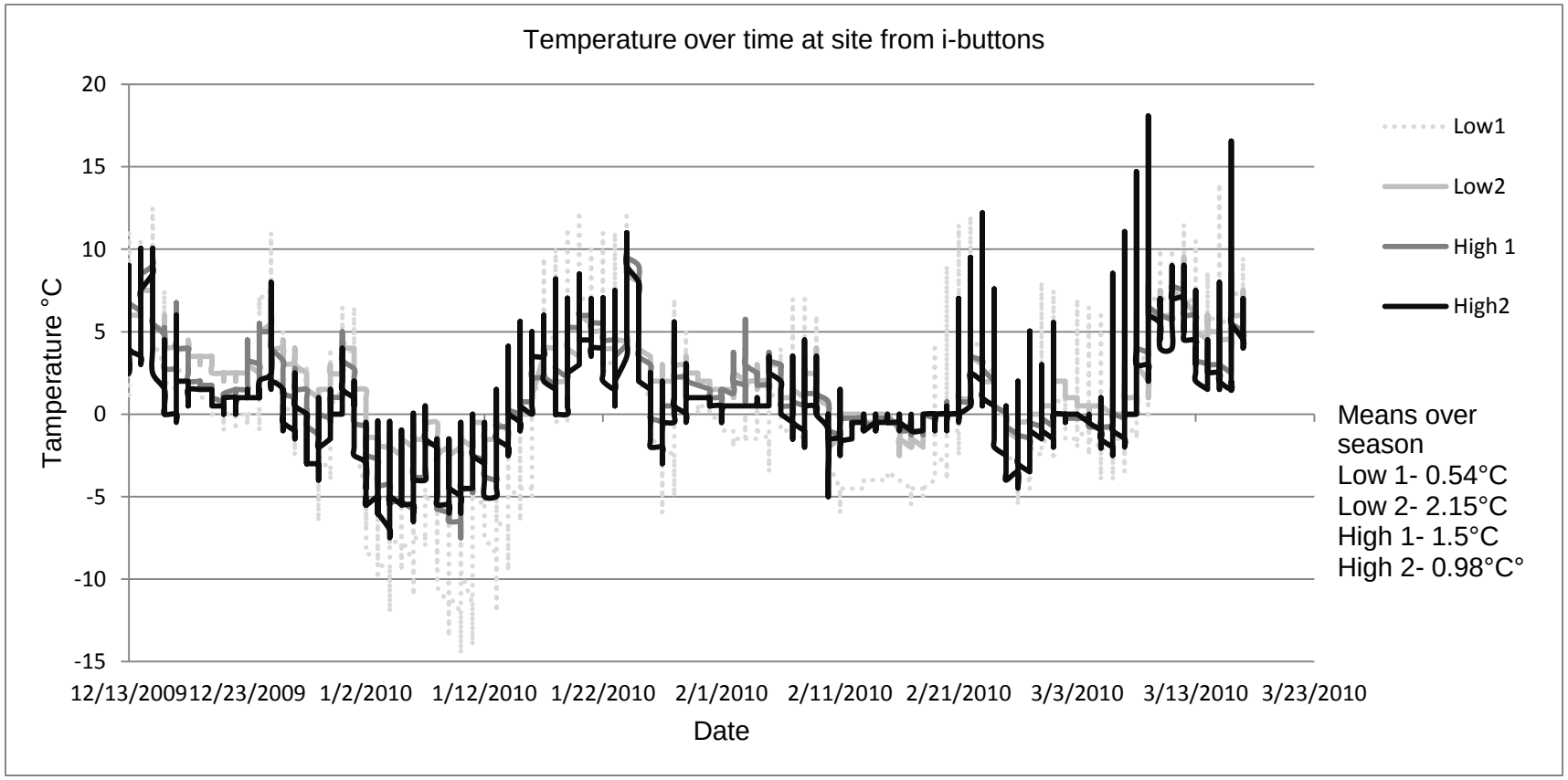

Figure 3. I-button readings (°C) from December 2009-March 2010 showing temperatures in Celcius. Two i-buttons were placed in each of the two gardens at the low and high elevation.

The plants were removed from the field and placed into the greenhouse nearing the end of their lifecycle, after siliques began to form but before they fully matured to prevent seed loss into the field. Some plants were still blooming when transferred into the greenhouse. *Alliaria petiolata* is known to be self-compatible for pollination, however, I noticed visiting pollinators in the field. Therefore, flowers that remained open in the greenhouse were manually pollinated with a paintbrush to ensure pollination (Durka et al. 2005). The plants were watered as needed, usually every day, after their return to the greenhouse. By the end of the field growth period, some of the plants in the low 1 garden had grown quite vigorously and their roots grew out of their peat pots and into the surrounding soil. Because of this, transfer from the field to the greenhouse caused wilting and flower death in some of these plants.

The end measurements were made in the greenhouse and lab starting in May, 2010, and included length of plant, number of leaves, number of stalks, survival, above-ground and below-ground mass, mass of seeds produced, number of siliques, number of seeds per silique, and approximate number of lost seeds. The number of seeds on a smaller sampling of plants were counted to calculate the average mass per seed. Because of *A. petiolata's* explosive dehiscence, some siliques lost seeds into the greenhouse. Number of seeds lost was measured by counting seed divots in each dehisced silique. The average mass per seed was used to calculate the approximate mass of seeds that were lost in the greenhouse. Seeds per silique was also counted on a smaller sampling of plants. The stalk length measurements were completed with a measuring tape. Number of leaves, stalks, and siliques were counted by hand. Numbers of leaves were counted as number per plant, though plants with no leaves were not computed into the average. The above-ground and below-ground parts were separated, soil was removed from the roots, and the plants were allowed to fully dry before being weighed. Initially the plants were placed in the oven for drying but the length of

processing time meant that all plants had time to reach similar ambient humidity found in the climate controlled laboratory before measurement. Filled seeds were separated from the above-ground part by winnowing and sifting after drying, but unfilled seeds were left in the mass of the above-ground parts. Number of seeds were counted to weigh and compute average mass per seed. This approximate seed mass was multiplied by the number of lost seed divots and then was added to seed mass measured for each plant. The labeling on the tags was not adequate upon transfer to the greenhouse and the two high elevation gardens were indistinguishable, so both low plots were combined together, and both high plots were combined together for analysis. Sample sizes vary due to many reasons. Zeroes as total for any plant were omitted for analysis of number of leaves at end and number of siliques. Plants with no leaves were not included in the herbivory analysis. Data recording was lacking on occasion due to misinformation by volunteers or mistakes and some fields had to be omitted (eg. Number of leaves per rosette). In all measurements by the end, data was omitted because some plant tags became faded and unreadable and population was unknown.

Germination Experiment

Alliaria petiolata seeds for the germination experiment were collected in the summer of 2010 by myself and two volunteers, from three regions and 5 populations: Cleveland, Ohio (Rocky River Metroparks; Lakewood, Ohio), New York (Bronx-NYB, Ossining-NYT and Cold Spring-NYH) and Asheville, North Carolina (Carrier Park) (Table 2). Ripe, dried siliques were placed into large bags, seeds were loosened from the siliques, and the fluffy chaff was removed above the heavier seeds. Seeds from each population were placed in a paper envelope, allowed to dry, and stored in an air-conditioned and heated lab.

In late January, 2011, a uniform scoop of Fafard™ seed starting mix was placed into 5.5cm plastic petri plates with a filter paper liner. Each petri plate received 30 garlic

mustard seeds: 19 plates were prepared with the NC seeds, 11 plates were prepared with the OH seeds, 9 plates with NYB, 6 plates with NYH and 15 plates with NYT. The number of plates varied due to seed limitation. The prepared plates were divided among three cold stratification treatments. All of the seeds were misted with distilled water and stacked on trays in a refrigerator kept at 3˚C.

Plates were assigned to three cold stratification time lengths: short (1584 hours), medium (1920 hours), and long (2400 hours). During cold treatment, plates were kept in the dark unless they were being observed. They were inspected weekly for water needs and misted if necessary, and the tray was rotated in the refrigerator. In mid-March, mold was observed on some of the plates, and these were removed from the experiment.

At the end of each experimental stratification period, plates in that treatment were removed from the refrigerator and placed on a windowsill of the climate controlled lab Plates were shuffled to reduce differences in light availability. Germinants were counted weekly and identified by emergence of the radicle. After 100 days, all plates were out of the refrigerator; germinants were removed from dishes, and remaining seeds were counted and allowed to continue to germinate for another 5 weeks until the end of the experiment at 147 days.

Statistical Analyses

Common Garden Experiment

Both the winter and end data comparisons among source-populations from the common garden study were analyzed with a one way ANOVA. Where appropriate, multiple comparisons were made among populations using Tukey's procedure to protect experiment-wise error rates. I also performed a two way ANOVA with elevation and population as factors and their interaction. The between elevations data were analyzed with a one way between groups ANOVA. The survival data were analyzed with a logistic regression. All analyses were conducted in R (R Project, Vienna, Austria).

The data from the high gardens, and the low gardens were pooled together and then were analyzed separately for each of the plant traits measured. Patterns were investigated by looking at means of trait measurements and post-hoc order of comparisons, sorting north to south in Excel (2010), using computed equivalent bioclimate using Hopkin's bioclimatic law (Hopkins 1920). Hopkins Bioclimatic law states that climbing 122 m up in elevation is equivalent to moving 1° N latitude. The site elevation was divided by 122 m then that factor was added to the site's true latitude, to compute the equivalent bioclimate. Patterns were also investigated with sorting highest to lowest means, north to south and by coldest to warmest using number of frost-free days and plant hardiness zones (Table 1). The pooled high and low gardens were compared to look for differing responses within the species as a whole between elevations.

Table 1. Source populations for the common garden experiment, showing zip code, elevation, USDA plant hardiness zone, mean annual temperature, latitude, average frost free days per year and computed bioclimate. Frost free days were from NOAA climate normals (2012) with the 50% last frost free day probability at 2.2°C. Plant zones were identified with the USDA interactive plant hardiness zone map (USDA Plant Hardiness Zones 2012). Bioclimate was calculated using Hopkins Bioclimatic Law (Hopkins 1920) *There are no climate normals calculated for Peaks of Otter. The nearest station in Bedford, Virginia is at 297m with 170 Frost Free days, and likely has a significantly different climate. Historical data for the station encompassing the years 1943 to 1976, is what constitutes the average annual temperature in this table

Source Location	Code	Elev. (m)	Plant zone	Frost Free days	Mean Annual Temperature	°North Latitude	°North Bioclimate
Warriors Path State Park, TN 37663	TN	381	7a	162	13.2	36.494	39.617
Antietam National Battlefield, MD 21782	MD1	113	6b	151	11.6	39.475	40.401
Carrier Park, Asheville, NC 28806	NC	603	7a	177	13.1	35.565	40.508
Clifton Forge, VA 24422	VA2	335	7a	151	12.6	37.815	40.561
Sky Meadows State Park, VA 20144	VA3	250	7a/6b	171	11.6	38.988	41.037
Fort Indiantown Gap, PA 17038	PA2	150	6b	169	10.9	40.424	41.654
Catoctin Mountain Park, MD 21788	MD2	262	6b	164	11.4	39.636	41.784
Calder Center, Armonk, NY 10504	NY	192	7a/6b	165	11.5	41.130	42.704
Peaks of Otter, VA 24523	VA1	775	6b	*	10.8*	37.448	43.800
Caledonia State Park, PA 17222	PA1	480	6b	162	11.5	39.911	43.845
Field-High, Highlands, NC 28741	HI	1190	6a/6b	147	11.1	35.139	44.893
Field-Low, Franklin, NC 28734	LOW	670	7a	147	12.6	35.171	40.663

Germination Experiment

Germination rates among populations over time were analyzed with a one way repeated measures ANOVA, treating the repeated measures as random variables in a mixed model, using the NLME package in R. The total germination among populations by the experiment's end was analyzed using one way ANOVA.

Table 2. Source populations for the cold stratification germination study showing the number of frost free days, mean annual temperature, frost free days, USDA plant zone, elevation, latitude and computed bioclimate. Mean annual temperature and frost free days were from the NOAA Climate Normals Data (2012), using 50%/ 2.2°C frost free data, and USDA plant hardiness zones from USDA Interactive Plant Hardiness Zone Map (2012). Bioclimate was computed with Hopkins Bioclimatic law using decimal latitude and elevation.

Location	Code	Mean Annual Temperature	Frost Free days	Plant Zone	Elevation	°North Latitude	°North Bioclimate
Cleveland, OH 44107	OH	10.1°	165	6b	215	41.479	43.241
Cold Spring, NY 10516	NYH	10.6˚	159	6b	33	41.429	41.699
Ossining, NY 10562	NYI	11.5°	156	7a/6b	49	41.153	41.555
Bronx, NY 10458	NYB	12.9˚	208	7a/7b	20	40.898	41.062
Asheville, NC 28806	NC	13.1°	177	7a	603	35.565	40.508

RESULTS

Common Garden Experiment

Winter Measurements Between Elevations

Plant growth, as measured by number of leaves per plant, was greater in the low gardens. Plants in low elevation gardens also had a greater percentage of leaves with evidence of herbivory (Table 3). Winter season odds of survival did not differ between high and low common gardens (Table 3).

Table 3. Mean winter growth and survival compared between high and low elevation gardens. Means and ± one standard deviation are shown.

Mean number of leaves per rosette $p < 0.0001$		
Site	Mean	N
HIGH	3.87 ±3.19	305
LOW	8.53 ±6.22	191
Odds of survival, p=0.0980		
Site	Mean	N
HIGH	0.91 ±0.28	305
LOW	0.95 ±0.21	208
Mean number of leaves showing herbivory/total number of leaves, $p < 0.0001$		
Site	Mean	N
HIGH	0.15 ±0.28	240
LOW	0.37 ±0.37	167

End of Experiment Measurements Between Elevations

Odds of survival to the end of the second season were greater in the low gardens. Reproduction output, as measured by approximate seed production and total number of mature siliques was greater in the low gardens (Table 4). Plant growth, as measured by above-ground biomass, below-ground biomass, longest stalk length, total number of stalks, and total number of leaves (Table 4) was greater in the low elevation gardens. Reproduction failure, as measured by number of aborted siliques per total was greater in the low elevation gardens. Seeds per siliques, did not differ between pooled high vs. low garden plants (Table 4).

Table 4. Means of survival, growth, and reproduction traits at the end of the second growing season in high and low elevation common gardens. Means and ± one standard deviation are shown.

Odds of survival $p < 0.0001$			Mean number of leaves, $p < 0.0001$		
Site	Mean	N	Site	Mean	N
HIGH	0.69 ±0.46	287	HIGH	30.6 ±17.79	194
LOW	0.97 ±0.17	176	LOW	49.99 ±32.84	172
Mean above-ground biomass (g) $p < 0.0001$			Mean below-ground biomass (g), $p < 0.0001$		
Site	Mean	N.	Site	Mean	N
HIGH	0.98 ±0.62	193	HIGH	0.14 ±0.11	204
LOW	2.97 ±2.13	166	LOW	0.52 ±0.46	173
Mean number of stalks; $p < 0.0001$			Longest stalk in cm, $p < 0.0001$		
Site	Mean	N	Site	Mean	N
HIGH	1.22 ±0.58	209	HIGH	47.8 ±18.23	204
LOW	1.99 ±1.58	175	LOW	68.25 ±18.2	171
Approximate seed production per plant, mass in grams $p < 0.0001$			Mean number of seeds per silique, $p= 0.9300$		
Site	Mean	N	Site	Mean	N
HIGH	0.28 ±0.19	187	HIGH	8.46 ±4.04	101
LOW	0.41 ±0.34	153	LOW	8.40 ±3.92	58
Number of siliques $p < 0.0001$			Aborted siliques per total number of siliques, $p < 0.0001$		
Site	Mean	N	Site	Mean	N
HIGH	15.91 ±11.85	210	HIGH	0.41 ±0.26	187
LOW	23.86 ±18.98	177	LOW	0.52 ±0.28	169

Winter Measurements Among Populations Within Gardens

Results presented below investigate differences using equivalent bioclimates as seen in Table 1. Results were also investigated differences when sorted by elevation, frost-free dates, north to south, and plant hardiness zones (Table 1), but these revealed no trends.

Odds of survival differed among populations in the high elevation gardens, but did not align with the equivalent bioclimate, and results did not differ in the low gardens (Table 5). The population x elevation analysis of survival was not significant. Among the populations of the gardens at both elevation, VA2 had full survival to winter, while PA1and TN had the poorest survival overall. The TN sample size is too small to be reliable in the low gardens and must be omitted (Table 5). Plant growth, as measured by leaves per rosette differed among populations but did not align with the equivalent bioclimate in either the high or low elevation gardens (Table 6). The population x elevation analysis of leaves per rosette was significant. No differences among populations were found for herbivory, computed with number of leaves showing herbivory per total number of leaves. The population x elevation interactions were not significant for herbivory (Table 7).

Table 5. Means and comparison of odds of survival after the first growing season among populations in the common gardens. Populations are listed from equivalent north to south using computed bioclimate. Sample size (number of identifiable plants) is indicated with "N". There were no differences for pairwise comparisions at either elevation. Means and ± one standard deviation are shown. Degrees of freedom for among populations ANOVA and p-value for population x elevation are listed at the bottom of the table.

High pooled, p=0.005			Low pooled, p=0.300		
Site	Mean	N	Site	Mean	N
PA1	0.88 ±0.34	32	PA1	0.90 ±0.31	39
VA1	0.90 ±0.31	30	VA1	1.00	33
NY	0.90 ±0.30	31	NY	1.00	14
MD2	0.94 ±0.25	32	MD2	0.95 ±0.22	20
PA2	0.97 ±0.17	33	PA2	0.96 ±0.20	24
VA3	0.94 ±0.25	31	VA3	1.00	9
VA2	1.00	28	VA2	1.00	8
NC	0.90 ±0.30	31	NC	0.97 ±0.17	33
MD1	1.00	31	MD1	0.92 ±0.28	24
TN	0.69 ±0.47	26	TN	0.75 ±0.50	4
Df=9	population x elevation		p=0.200		

Table 6. Means and comparison of number of leaves per rosette, among populations in the common garden experiment. Populations are listed from equivalent north to south using computed bioclimate. Sample size (number of plants with leaves) indicated with "N". Sample sizes that are too small for meaningful data are marked with a single asterisk. Means with the same letter under "Signif." do not differ significantly (P > 0.05). Means and ± one standard deviation are shown. Degrees of freedom for among populations ANOVA and p-value for population x elevation are listed at the bottom of the table.

	High, p=0.0020				Low, p=0.0020		
Site	Mean	Signif.	N	Site	Mean	Signif.	N
PA1	5.06 ±5.10	a	32	PA1	6.72 ±4.45	a	39
VA1	2.43 ±1.83	b	30	VA1	7.68 ±3.89	a	31
NY	3.74 ±2.79	ab	31	NY	8.54 ±3.80	ab	13
MD2	4.44 ±3.11	ab	32	MD2	7.05 ±6.11	a	19
PA2	4.33 ±2.78	ab	33	PA2	8.87 ±5.99	ab	23
VA3	2.45 ±1.91	b	31	VA3	8.00 ±5.45	ab	7
VA2	4.71 ±2.55	ab	28	VA2	14.17 ±3.19	ab	6
NC	4.03 ±3.43	ab	31	NC	12.70 ±10.01	b	30
MD1	4.52 ±3.05	ab	31	MD1	6.67 ±3.32	a	21
TN	2.77 ±3.02	ab	26	TN	9.50 ±13.44	ab	2*
	Df=9	population x elevation			p=0.0001		

Table 7. Means and comparisons of leaves showing herbivory per total number of leaves, among populations in the common garden. Populations are listed from equivalent north to south using computed bioclimate. Sample size (number of plants with leaves) indicated with "N". Sample sizes that are too small for meaningful data are marked with a single asterisk. There were no differences for pairwise comparisions at either elevation. Means and ± one standard deviation are shown. Degrees of freedom for among populations ANOVA and p-value for population x elevation are listed at the bottom of the table.

High pooled, p=0.200			Low pooled, p=0.800		
Site	Mean	N	Site	Mean	N
PA1	0.14 ±0.23	25	PA1	0.27 ±0.29	33
VA1	0.29 ±0.38	19	VA1	0.41 ±0.49	30
NY	0.21 ±0.35	25	NY	0.42 ±0.37	12
MD2	0.18 ±0.35	29	MD2	0.36 ±0.32	12
PA2	0.11 ±0.22	29	PA2	0.35 ±0.26	20
VA3	0.14 ±0.32	21	VA3	0.52 ±0.33	6
VA2	0.17 ±0.24	27	VA2	0.29 ±0.31	6
NC	0.09 ±0.23	25	NC	0.39 ±0.39	28
MD1	0.11 ±0.24	27	MD1	0.42 ±0.39	19
TN	0	13	TN	0.57	1*
Df=9	population x elevation		p=0.300		

End of Experiment Measurements Among Populations Within Gardens

Odds of survival (Table 8) differed among populations in the high elevation gardens only, and the population x elevation interactions were not significant. Within the populations of the gardens, there was 100% survival in VA2 at both elevations while MD1, PA2 and NY all had high survival in the high gardens and 100% survival in the low gardens (Table 8). Plant growth, as measured by number of stalks (Table 9) differed among populations in the low gardens and aligned with greater number of stalks in the more southern equivalent bioclimate. The interaction between population and elevation was significant for number of stalks as well. Above-ground (Table 10) and below-ground biomass (Table 11) showed differences among populations only for the high gardens but differences did not align with the equivalent bioclimate. The population x elevation interaction was significant in the above-ground biomass but not the below-ground biomass. Population MD2 ranked second, in above -and below-ground biomass in the high gardens when means were sorted highest to lowest among the other populations, and exhibited comparatively lesser biomass in the low gardens, ranked last. NY produced higher biomass at both elevations, ranked first in the high gardens for both above- and below-ground biomass, and in the low gardens NY ranged second in below-ground and third in above-ground biomass, if the small TN population is removed (Table 10 and 11). When results were sorted by elevation, only one trait showed relationships among populations with similar source elevation: number of stalks in the low gardens. Number of stalks showed clustering of similar means with all three sorting methods but none aligned linearly perfect (Table 9). No traits showed similarities or clustering among source populations when the results were sorted by latitude. Plant growth traits that showed no significant differences among populations were longest stalk length (Table 12), and final number of leaves (Table 13).

Reproductive output, measured by number of mature siliques (Table 14), and approximate seed production (Table 15), differed among populations but did not align with the equivalent bioclimate in high gardens. Both measurements of reproductive output showed significant interactions among population and elevation (Table 14 & 15). Reproductive failure, measured by the number of aborted siliques per total siliques (Table 16), differed among populations with no alignment to the equivalent bioclimate in either low or high elevation gardens, and the population x elevation interaction was not significant. Seeds per silique was the only trait that did not differ among populations, nor was the population x elevation interaction significant (Table 17). The NY population produced a greater number of siliques at both elevations, while MD1 and NC produced fewer siliques at both elevations. However, PA2 produced a high number among populations when sorted by means for number of siliques in the high gardens, but fewer when sorted by means in the low gardens.

Table 8. Means and comparison of odds of survival by end among populations in the common garden. Populations are listed from equivalent north to south using computed bioclimate. Sample size (number of plants in the garden) indicated with "N". Sample sizes that are too small for meaningful data are marked with a single asterisk. Means with the same letter under "Signif." do not differ significantly (P > 0.05). There were no differences for pairwise comparisions at the low elevation. Means and ± one standard deviation are shown. Degrees of freedom for among populations ANOVA and p-value for population x elevation are listed at the bottom of the table.

	High pooled, p< 0.0001			Low pooled, p= 0.4000		
Site	Mean	Signif.	N	Site	Mean	N
PA1	0.68 ±0.48	bc	28	PA1	1.00	30
VA1	0.27 ±0.45	c	26	VA1	0.93 ±0.26	29
NY	0.89 ±0.31	ab	28	NY	1.00	13
MD2	0.77 ±0.43	ab	30	MD2	0.93 ±0.27	14
PA2	0.90 ±0.31	ab	29	PA2	1.00	20
VA3	0.31 ±0.47	c	26	VA3	1.00	8
VA2	0.89 ±0.32	ab	27	VA2	1.00	8
NC	0.67 ±0.48	bc	27	NC	0.93 ±0.27	27
MD1	0.93 ±0.26	b	28	MD1	1.00	20
TN	0.48 ±0.51	ac	27	TN	1.00	2*
	Df=35	population x elevation			p=0.9000	

Table 9. Means and comparison of number of stalks among populations in the common garden. Populations are listed from equivalent north to south using computed bioclimate. Sample size (total number of surviving plants) indicated with "N". Sample sizes that are too small for meaningful data are marked with a single asterisk. Means with the same letter under "Signif." do not differ significantly (P > 0.05). There were no differences for pairwise comparisions in the high elevation. Means and ± one standard deviation are shown. Degrees of freedom for among populations ANOVA and p-value for population x elevation are listed at the bottom of the table.

	High pooled, p=0.08			Low pooled, p=0.03		
Site	Mean	N	Site	Mean	Signif.	N
PA1	1.05 ±0.49	22	PA1	1.48 ±1.15	a	29
VA1	1.57 ±0.98	7	VA1	1.67 ±1.12	ab	30
NY	1.36 ±0.76	25	NY	1.62 ±0.96	ab	13
MD2	1.00 ±0.28	26	MD2	1.47 ±1.19	ab	15
PA2	1.19 ±0.49	26	PA2	1.95 ±1.61	ab	20
VA3	1.13 ±0.35	8	VA3	2.25 ±1.91	ab	8
VA2	1.15 ±0.46	26	VA2	2.71 ±1.50	ab	7
NC	1.40 ±0.68	20	NC	2.80 ±2.22	b	25
MD1	1.38 ±0.70	26	MD1	2.14 ±1.31	ab	21
TN	1.08 ±0.28	13	TN	3.50 ±2.12	ab	2*
	Df=9		population x elevation	p=0.05		

Table 10. Means and comparison of above-ground biomass in grams among populations in the common garden, end measurements. Populations are listed from equivalent north to south using computed bioclimate. Sample size (plants with above-ground parts) indicated with "N". Sample sizes that are too small for meaningful data are marked with a single asterisk. Means with the same letter under "Signif." do not differ significantly (P > 0.05). There were no differences for pairwise comparisions in the low elevation. Means and ± one standard deviation are shown. Degrees of freedom for among populations ANOVA and p-value for population x elevation are listed at the bottom of the table.

	High pooled, p=0.0006				Low pooled, p= 0.09	
Site	Mean	Signif.	N	Site	Mean	N
PA1	0.72 ±0.53	b	19	PA1	2.52 ±1.94	30
VA1	0.41 ±0.26	b	7	VA1	2.52 ±1.52	27
NY	1.34 ±0.50	a	25	NY	3.21 ±1.44	13
MD2	1.14 ±0.63	ab	24	MD2	1.79 ±0.75	13
PA2	1.07 ±0.58	ab	26	PA2	2.67 ±2.05	20
VA3	0.71 ±0.85	ab	9	VA3	2.84 ±1.44	8
VA2	0.74 ±0.54	b	25	VA2	3.75 ±1.96	8
NC	0.87 ±0.41	ab	19	NC	3.73 ±2.72	25
MD1	0.99 ±0.73	ab	26	MD1	3.40 ±2.53	20
TN	1.11 ±0.62	ab	13	TN	5.34 ±1.95	2*
	Df=9			population x elevation	p=0.009	

Table 11. Means and comparison of below-ground biomass in grams among populations in the common garden. Populations are listed from equivalent north to south using computed bioclimate. Sample size (plants that produced roots) indicated with “N”. Sample sizes that are too small for meaningful data are marked with a single asterisk. Means with the same letter under “Signif.” do not differ significantly (P > 0.05). There were no differences for pairwise comparisions in the low elevation. Means and ± one standard deviation are shown. Degrees of freedom for among populations ANOVA and p-value for population x elevation are listed at the bottom of the table.

	High pooled, p=0.001			Low pooled, p= 0.200		
Site	Mean	Signif.	N	Site	Mean	N
PA1	0.10 ±0.08	b	20	PA1	0.54 ±0.54	29
VA1	0.06 ±0.05	b	7	VA1	0.37 ±0.27	27
NY	0.20 ±0.11	a	26	NY	0.65 ±0.42	14
MD2	0.15 ±0.09	ab	23	MD2	0.24 ±0.16	13
PA2	0.14 ±0.10	ab	26	PA2	0.56 ±0.47	20
VA3	0.12 ±0.12	ab	9	VA3	0.46 ±0.39	8
VA2	0.11 ±0.08	b	24	VA2	0.67 ±0.69	8
NC	0.20 ±0.16	ab	20	NC	0.53 ±0.38	26
MD1	0.11 ±0.07	b	26	MD1	0.63 ±0.60	21
TN	0.15 ±0.12	ab	13	TN	0.83 ±0.41	2*
	Df=9	population x elevation			p=0.100	

Table 12. Means and comparison of longest stalk in centimeters, among populations in the common garden. Populations are listed from equivalent north to south using computed bioclimate. Sample size (plants that grew stalks) indicated with “N”. Sample sizes that are too small for meaningful data are marked with a single asterisk. There were no differences for pairwise comparisions at either elevation. Means and ± one standard deviation are shown. Degrees of freedom for among populations ANOVA and p-value for population x elevation are listed at the bottom of the table.

High pooled, p=0.06			Low pooled, p= 0.80		
Site	Mean	N	Site	Mean	N
PA1	42.25 ±16.29	20	PA1	67.88 ±17.89	29
VA1	32.77 ±23.08	7	VA1	71.39 ±19.41	28
NY	53.90 ±14.17	24	NY	71.0 2±16.37	13
MD2	46.29 ±17.98	26	MD2	68.91 ±14.75	14
PA2	50.09 ±17.01	25	PA2	62.73 ±16.37	20
VA3	46.53 ±26.80	8	VA3	70.28 ±22.57	8
VA2	46.76 ±19.06	25	VA2	73.80 ±14.17	7
NC	42.83 ±16.84	20	NC	67.52 ±17.91	24
MD1	47.37 ±15.45	26	MD1	67.50 ±17.92	21
TN	53.02 ±21.83	13	TN	85.50 ±0.71	2*
	Df=9		population x elevation		p=0.40

Table 13. Means and comparison of total leaves per plant among populations in the common garden, end measurements. Populations are listed from equivalent north to south using computed bioclimate. Sample size (plants that produced leaves) indicated with "N". Sample sizes that are too small for meaningful data are marked with a single asterisk. There were no differences for pairwise comparisions at either elevation. Means and ± one standard deviation are shown. Degrees of freedom for among populations ANOVA and p-value for population x elevation are listed at the bottom of the table.

High pooled, p=0.20			Low pooled, p= 0.06		
Site	Mean	N	Site	Mean	N
PA1	27.86 ±17.64	21	PA1	41.14 ±26.09	29
VA1	20.29 ±10.69	7	VA1	41.10 ±22.10	29
NY	36.72 ±25.46	25	NY	49.85 ±18.28	13
MD2	30.44 ±13.33	25	MD2	39.00 ±17.93	14
PA2	32.13 ±17.02	24	PA2	56.35 ±49.19	20
VA3	16.29 ±6.87	7	VA3	53.25 ±43.67	8
VA2	34.92 ±22.54	24	VA2	50.00 ±18.43	7
NC	33.74 ±14.14	19	NC	67.55 ±39.18	29
MD1	29.65 ±15.10	26	MD1	47.90 ±31.53	21
TN	24.00 ±11.32	12	TN	75.50 ±41.72	2*
	Df=9		population x elevation		p=0.20

Table 14. Means and comparison of number of mature siliques produced per plant among populations in the common garden. Populations are listed from equivalent north to south using computed bioclimate. Sample size (number of plants that produced siliques) number indicated with "N". Sample sizes that are too small for meaningful data are marked with a single asterisk. Means with the same letter under "Signif." do not differ significantly (P > 0.05). Means and ± one standard deviation are shown. Degrees of freedom for among populations ANOVA and p-value for population x elevation are listed at the bottom of the table.

	High pooled, p< 0.0001				Low pooled, p= 0.0005		
Site	Mean	Signif.	N	Site	Mean	Signif.	N
PA1	11.33 ±7.88	bc	21	PA1	22.69 ±16.70	ab	29
VA1	10.00 ±8.65	ac	6	VA1	33.13 ±24.40	a	30
NY	22.31 ±13.24	a	26	NY	37.54 ±17.03	a	13
MD2	18.35 ±13.22	ac	26	MD2	27.21 ±14.83	ab	14
PA2	20.11 ±11.84	ab	27	PA2	14.00 ±14.75	b	20
VA3	17.88 ±16.41	ac	8	VA3	26.13 ±13.94	ab	8
VA2	13.88 ±11.10	ac	26	VA2	31.00 ±20.74	ab	7
NC	8.70 ±5.66	c	20	NC	16.07 ±13.63	b	28
MD1	10.35 ±7.01	bc	26	MD1	17.71 ±18.75	ab	21
TN	21.38 ±15.27	ac	13	TN	40.00 ±7.07	ab	2*
	Df=9	population x elevation			p=0.0080		

Table 15. Means and comparison of approximate seed production (g), among populations in the common garden; values include estimates for lost seeds. Populations are listed from equivalent north to south using computed bioclimate. Sample size (number of plants that produced seeds) number is indicated with "N". Sample sizes that are too small for meaningful data are marked with a single asterisk. Means with the same letter under "Signif." do not differ significantly (P > 0.05). There were no differences for pairwise comparisions in the low elevation. Means and ± one standard deviation are shown. Degrees of freedom for among populations ANOVA and p-value for population x elevation are listed at the bottom of the table.

	High pooled,	p< 0.0001			Low pooled, p= 0.2000	
Site	Mean	Signif.	N	Site	Mean	N
PA1	0.13 ±0.15	acde	29	PA1	0.37 ±0.32	33
VA1	0.03 ±0.08	e	26	VA1	0.34 ±0.41	32
NY	0.29 ±0.22	ab	30	NY	0.48 ±0.43	16
MD2	0.26 ±0.26	bc	32	MD2	0.39 ±0.32	15
PA2	0.30 ±0.22	b	31	PA2	0.16 ±0.19	20
VA3	0.08 ±0.20	de	26	VA3	0.40 ±0.33	8
VA2	0.21 ±0.19	bd	28	VA2	0.59 ±0.48	8
NC	0.11 ±0.14	cde	30	NC	0.31 ±0.32	28
MD1	0.14 ±0.12	be	28	MD1	0.19 ±0.27	21
TN	0.16 ±0.23	be	27	TN	0.59 ±0.36	2*
	Df=9	population x elevation			p=0.0010	

Table 16. Means and comparison of total number of aborts divided by total number of siliques per plant, aborted and ripe, among populations in the common garden. Populations are listed from equivalent north to south using computed bioclimate. Sample size number indicated with "N". Sample sizes that are too small for meaningful data are marked with a single asterisk. Means with the same letter under "Signif." do not differ significantly (P > 0.05). Means and ± one standard deviation are shown. Degrees of freedom for among populations ANOVA and p-value for population x elevation are listed at the bottom of the table.

	High pooled, p< 0.0001				Low pooled, p= 0.0200		
Site	Mean	Signif.	N	Site	Mean	Signif.	N
PA1	0.52 ±0.28	ac	19	PA1	0.50 ±0.26	ab	29
VA1	0.32 ±0.27	ab	5	VA1	0.47 ±0.26	ab	28
NY	0.36 ±0.15	bc	25	NY	0.46 ±0.16	ab	13
MD2	0.42 ±0.22	ab	25	MD2	0.52 ±0.26	ab	14
PA2	0.29 ±0.15	b	25	PA2	0.49 ±0.34	ab	20
VA3	0.25 ±0.24	bc	7	VA3	0.28 ±0.15	b	7
VA2	0.30 ±0.19	bc	23	VA2	0.38 ±0.28	ab	7
NC	0.58 ±0.36	a	20	NC	0.62 ±0.28	ab	28
MD1	0.60 ±0.26	a	26	MD1	0.65 ±0.27	a	21
TN	0.26 ±0.12	bc	12	TN	0.24 ±0.03	ab	2*
	Df=9	population x elevation			p=0.8000		

Table 17. Means and comparison of number of seeds per silique among populations in the common garden. Populations are listed from equivalent north to south using computed bioclimate. Sample size number indicated with “N”. Sample sizes that are too small for meaningful data are marked with a single asterisk. There were no differences for pairwise comparisions at either elevation. Means with the same letter under “Signif.” do not differ significantly (P > 0.05). Means and ± one standard deviation are shown. Degrees of freedom for among populations ANOVA and p-value for population x elevation are listed at the bottom of the table.

High pooled, p=0.4			Low pooled, p= 0.4		
Site	Mean	N	Site	Mean	N
PA1	8.56 ±4.19	8	PA1	6.99 ±2.49	10
VA1	8.47 ±4.98	5	VA1	8.24 ±3.12	12
NY	10.83 ±4.52	13	NY	10.5 ±0.24	2
MD2	8.92 ±4.44	11	MD2	10.07 ±2.12	5
PA2	8.27 ±3.60	15	PA2	7.48 ±3.80	7
VA3	5.17 ±3.64	5	VA3	11.67 ±0.76	3
VA2	8.23 ±2.88	13	VA2	10.75	1*
NC	6.64 ±4.52	7	NC	9.91 ±5.52	9
MD1	8.45 ±4.13	14	MD1	6.62 ±5.37	9
TN	8.27 ±3.82	10	TN	na	0*
	Df=8		population x elevation		p=0.3

Germination Experiment

Populations differed in cold stratification requirements, but the differences did not align with an equivalent bioclimatic gradient. After 2400 hours of cold stratification, all but NYH and NYB had greater than 90% germination (Fig. 4, Table 18). Two populations showed >20% germination with 1584 hours of cold stratification: OH, 23%; NYH, 29%, while the rest were lower, OH, 23%; NYH, 29%; NC and NYT, 2%; NYB 1% (Fig. 4, Table 20). The medium length season of 1920 hours showed varying amounts of germination among the populations: NYH, 86%; NC, 79%; NYT, 72%; NYB, 58%; OH, 50% (p=0.0012).

Figure 4. Percent germination among populations for three season lengths: short-stratification season, 66 days (1584 hours), medium-stratification season, 80 days (1920 hours), and long-stratification season, 100 days (2400 hours). P values and numerical percentages are found in Table 18.

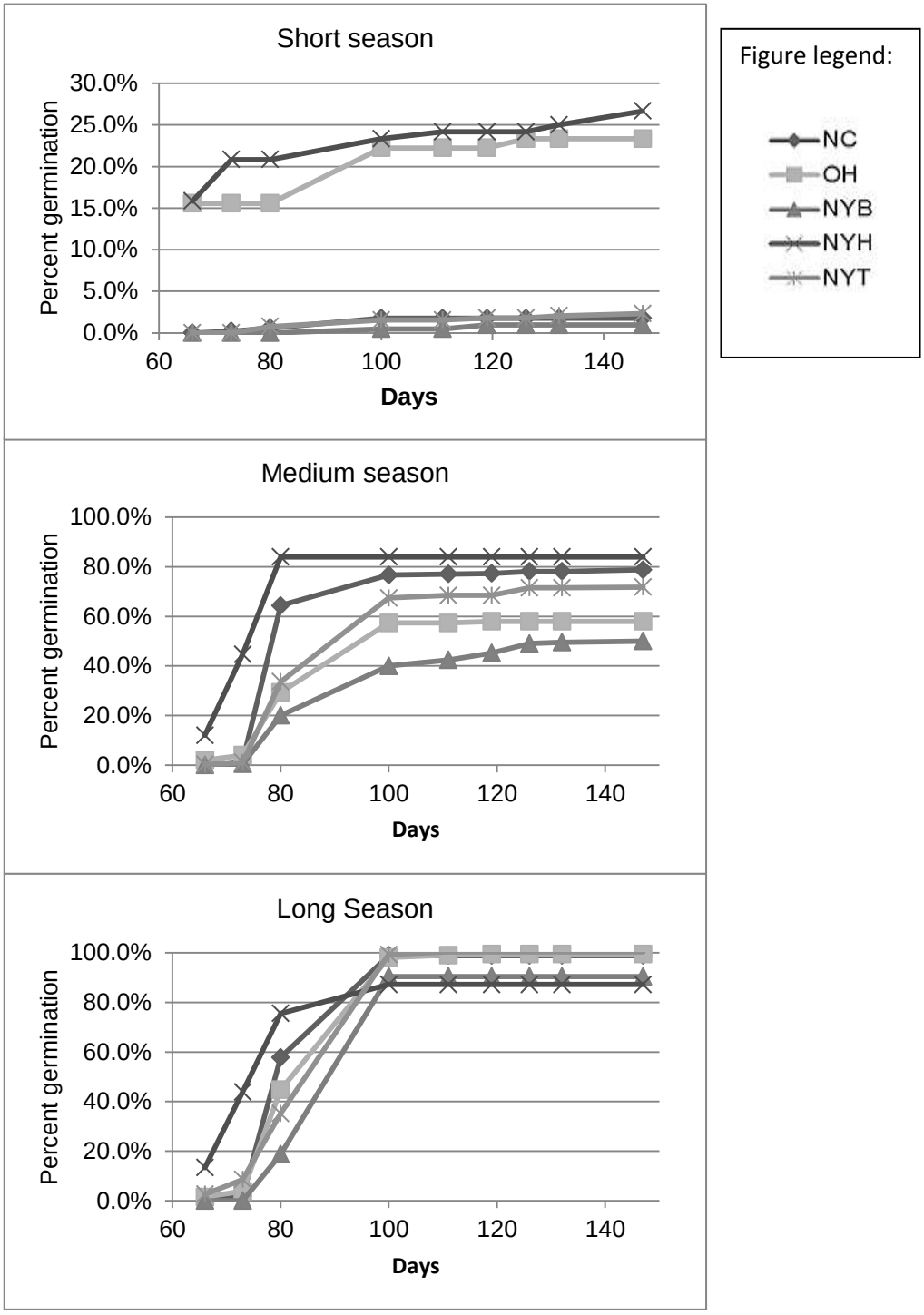

Table 18. Comparisons of proportion of germination over time for the cold-stratification study. Germinants were counted for three different season lengths and and at the end of the experimentl: 1584 chilling hours (66 days), 1920 chilling hours (80 days), and 2400 chilling hours (100 days) and at the end of 147 days. Sample sizes are indicated with "N." Means and ± one standard deviation are shown.

1584 chill hours

Site	66 days	80 days	100 days	147 days	N
NC	0	0.01 ±0.01	0.02 ±0.02	0.02 ±0.02	17
OH	0.16 ±0.19	0.16 ±0.19	0.22 ±0.30	0.23 ±0.30	3
NYB	0	0	0 ±0.01	0.01 ±0.02	7
NYH	0.19 ±0.04	0.25 ±0.1	0.26 ±0.11	0.29 ±0.09	4
NYT	0	0.01 ±0.03	0.02 ±0.04	0.02 ±0.05	13
p value	< 0.0001	< 0.0001	< 0.0001	0.32	

1920 chill hours

Site	66 days	80 days	100 days	147 days	N
NC	0	0.64 ±0.15	0.77 ±0.12	0.79 ±0.11	16
OH	0.02 ±0.03	0.29 ±0.19	0.57 ±0.38	0.58 ±0.38	5
NYB	0	0.20 ±0.16	0.40 ±0.16	0.50 ±0.17	7
NYH	0.17 ±0.09	0.84 ±0.06	0.86 ±0.08	0.86 ±0.08	5
NYT	0 ±0.01	0.34 ±0.16	0.67 ±0.13	0.72 ±0.12	13
p value	< 0.0001	< 0.0001	0.00011	0.0012	

2400 chill hours

Site	66 days	80 days	100 days	147 days	N
NC	0	0.58 ±0.20	0.99 ±0.05	0.99 ±0.05	15
OH	0.01 ±0.03	0.45 ±0.18	0.98 ±0.09	1.00 ±0.10	7
NYB	0	0.19 ±0.12	0.90 ±0.23	0.9 0 ±0.23	8
NYH	0.13 ±0.19	0.76 ±0.15	0.87 ±0.11	0.87 ±0.11	6
NYT	0.03 ±0.08	0.35 ±0.27	0.99 ±0.01	1.00 ±0.03	9
p value	0.011	< 0.0001	0.14	0.11	

DISCUSSION AND CONCLUSION

Over the last 150 years since its introduction point at Long Island, NY, *A. petiolata* has spread west to the prairies, south along the mid-Atlantic, and down the Appalachians. This spread has encompassed 15° latitude, from near Asheville, NC, at 670m elevation, to Quebec, at 50° latitude and 90m elevation. Outlier populations have been identified in Kennesaw, Ga, at N 34° and 450m and Fayetteville, NC at N 35° and 75m in elevation (EDDMAPS 2014).

Differences in survival within the high-elevation common gardens indicate differentiation among *A. petiolata* populations throughout the introduced range, from NY to Asheville. However, there is no clear pattern of adaptation with respect to equivalent bioclimate, latitude or elevation. Rather, survival of populations from VA2, MD1, NY, and PA2 was greater than the other populations at both high and low elevations, suggesting survival was affected by something other than the different climates in the common gardens.

Alliaria petiolata growth, when all populations were pooled (above and below ground biomass, leaf production, number of stalks per plant) differed between the high- and low-elevation gardens and on average was greater in the low elevation gardens. These results suggest that once established, *A. petiolata* plants would not be limited by mild winters and warm summers in sites at least as far south as Asheville. Differences in number of stalks per plant among populations in the low-elevation gardens, and differences in below-ground biomass, above-ground biomass, and number of leaves per plant among populations in the high elevation gardens, collectively suggest population differentiation due to drift or founder effects, as there was no consistent pattern of differences.

Introduced *A. petiolata* has reduced genetic diversity compared to the native range, but the genetic differences between native and introduced populations is not great because it is a highly inbred, selfing, plant (Durka et. al 2005). With low genetic diversity, introduced species are prone to genetic drift and inbreeding depression (Sakai et al. 2001). Recently, founder effects, but not inbreeding depression, have been found in *A. petiolata* (Mullarkey et al. 2013). The Mullarkey et al. (2013) study found founder effects in rosette size of *A. petiolata*, though they were only apparent when the species was grown in an intraspecies competitive environment; my study did not test this as each plant had its own pot, but there were differences among populations in both the low and high elevation and there were significant interactions for population x elevation. While the mechanism is unknown, there are clearly differences in rosette size. In a study of *Cynoglossum officinale* L., Williams and Fishman (2014) found little evidence that inbreeding depression occurred, but that different alleles could be fixed into a population as well due to reduced genetic diversity within populations in the introduced range. Their results suggest that establishment, spread, and potentially adaptation of a species to a new range is not prevented by reduced genetic diversity. When invasive *Senecio inaequidens* DC. populations, from elevations of 5 to 1635 m, were grown together in a common garden, plants grown from seeds originating in the higher elevation were shorter and had lower biomass, indicating differentiation and potentially adaptation in growth after introduction (Monty and Mahy 2009). Population MD2, exhibited comparatively high above- and below-ground biomass in the high gardens, but exhibited comparatively lesser biomass in the low gardens, indicating variation and possible adaptation, to the higher elevation in this one population. In contrast, NY produced higher biomass at both elevations (ranked first in the high gardens for both above and below-ground biomass, second in below-ground and third in above-ground in the low gardens for biomass, if the small TN population is removed), which means the below-

ground biomass production for this source is not affected as strongly by environment. This seems to indicate that growth in some populations was less affected by the climate differences than others, and these populations would perform better in a novel environment. It may also be a genotype by environment interaction.

In a separate common garden study of *Senecio inaequidens,* using plants from differing elevations, seed mass production varied among populations with greater production in all populations in the warmer treatment (Monty et al. 2009). Similarly, the reproductive effort of *A. petiolata* was overall greater in the low elevation gardens but varied among populations in the high elevation gardens. Specifically, the northern population, PA2, produced more seeds in the high elevation gardens but produced comparatively less in the low gardens when means were ranked with the other populations, while NY produced comparatively high in both gardens. These results suggest some *A. petiolata* populations might be more tolerant of the more stressful winters or cooler summers of higher elevation sites such as Highlands, NC, at 1190m, or other areas with a cooler climate. Although unlikely, it is not inconceivable that seeds from populations like PA2 and NY could be transported to higher elevation sites and reproduce well.

A study of *Xanthium strumarium* L. (common cocklebur), a native, but weedy plant, found that northern populations flowered earlier when grown together in a common garden, but that there was a lot of variation among the populations in flowering phenology and a number of other traits (Wassom et. al. 2002). Evidence of adaptation was found in *X. strumarium* in regards to flowering phenology, but the evidence was not strong enough to support a specific regional ecotype, as there was a lot of variation found among all populations from north to south. I did not find much evidence for adaptation in *A. petiolata* but did find strong evidence for variation among populations for a number of traits.

Differences among populations in germination following lengths of 1584 hours, 1940 hours, and 2400 hours of cold treatments indicate that there are differences among populations of *A. petiolata* in its invasive range. All populations ranged between 87% and 100% germination after 2400 hours of cold treatment, which has been shown in previous research to be the stratification requirement for *A. petiolata* (Baskin and Baskin 1992), though NYH only increased germination 1% between the medium and long season treatments, and may not benefit from more than 1940 hours of cold stratification. However, even in the shortest season, two populations had >20% germination and if seeds from the NYH and OH populations were transported south, as possible along the I-81 corridor, germination could occur as far south as central Georgia, with an average of 1590 chilling hours (threshold of <7.5°C for accumulating chilling) (Agroclimate™ 2014). Previous studies of *A. petiolata* have found temperatures below <6°necessary for cold stratification to occur (Baskin and Baskin 1992, Raghu and Post 2008), so the <7.5°C threshold range may indicate a slightly more southern location than could provide appropriate cold stratification for *A. petiolata*. However, even the relatively low germination rate of 1%-26% for the shortest stratification season could allow for germination of *A. petiolata* as each plant can produce over 800 seeds (Nuzzo 2000), although it is possible the species may no longer be ecologically invasive with such low germination rates if seedling establishment rates are low.

There is some evidence of species evolving different germination requirements in their introduced range compared to their native range, such as suppressed germination at higher temperatures in *Cardamine hirsute* L., and higher and earlier rates of seedling emergence in *Rhododendron ponticum* L. (Walck et al. 2011). Few studies have examined germination in the native range of *A. petiolata*, though one study did find a range of cold stratification season lengths in the former Czechoslovakia (Lhotská 1975). It may be beneficial to look for evidence of adaptation in cold stratification between the

native and introduced ranges by doing a larger study on *A. petiolata* that utilizes seeds from more populations in both ranges.

In sum, *A. petiolata* generally responds with greater growth, reproduction and survival in the warmer environments, suggesting some amount of pre-adaptation to warmer climate. However, responses of some populations across the invasive range varied at different elevations and climates. Founder effects are due to the reduced number of allele frequencies from a small, isolated, introduced population that can allow for genetic drift (Matute 2013). Founder effects have been found in *A. petiolata* (Mullarkey et al. 2013), so perhaps some variation may be attributed to this phenomenon. This study cannot adequately determine whether plasticity is a factor in *A. petiolata*, as the genetic similarities and differences of the populations used are unknown. Generally clones, or at least siblings are used to ensure genetic similarities so that different responses are definitely attributable to plasticity and not genetic variation.

If seeds from populations that require less cold stratification to germinate than other populations, or from populations that are better at surviving in novel environments get transported to the edge *of A. petiolata's* range or into a protected microclimate, such as a north facing slope in the Piedmont, the species may be able to expand its range out of the southern Appalachians, as the population at Kennesaw Mountain in suburban Atlanta may indicate (personal observation). The variation among populations of *A. petiolata* supports the idea that it can continue to invade habitats within its current range, with some potential for expansion in the south. A changing climate ensures that it will continue to expand to higher elevations and in the north beyond its current range to central Quebec as temperatures in the northern forests increase (Welk et al. 2002, Walck et al. 2011).

LITERATURE CITED

Anderson RC, S Dhillion, T Kelley. 1996. Aspects of the ecology of garlic mustard (*Alliaria petiolata*) in central Illinois. Restoration Ecology 4: 81-91.

Baker HG. 1974. The evolution of weeds. Annual Review of Ecology and Systematics 5: 1-24.

Baskin JM, CC Baskin. 1992. Seed-germination biology of the weedy biennial *Alliaria petiolata*. Natural Areas Journal 12: 191–197.

Baskin JM, CC Baskin. 2001. Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. San Diego (Ca): Academic Press. 648 p

Baskin JM, CC Baskin. 2004. A classification system for seed dormancy. Seed Science Research, 14: 1-16

Bender MH, JM Baskin, CC Baskin. 2003. Seed germination ecology of *Polymnia canadensis* (*Asteraceae*), a monocarpic species of the North American temperate deciduous forest. Plant Ecology 168(2): 221-253.

Bossdorf O, D Prati, H Auge, B Schmid. 2004a. Reduced competitive ability in an invasive plant. Ecology Letters 7: 346–353.

Bossdorf O, S Schroder, D Prati, H Auge. 2004b. Palatability and tolerance to simulated herbivory in native and introduced populations of *Alliaria petiolata* (*Brassicaceae*). American Journal of Botany 91(6): 856-862.

Bossdorf O, A Lipowsky, D Prati. 2008. Selection of preadapted populations allowed *Senecio inaequidens* to invade Central Europe. Diversity and Distributions. 14: 676-685.

Byers DL, JA Quinn. 1998. Demographic variation in *Alliaria petiolata* (*Brassicaceae*) in 4 contrasting habitats. Journal of the Torrey Botanical Society. 125(2): 138-149.

Cavers PB, MI Heagy, RF Kokron. 1979. The Biology of Canadian Weeds 35: *Alliaria petiolata* (M. Bieb) Cavara and Grande. Canadian Journal of Plant Science. 59: 217-229.

Cipollini D. 2002. Variation in the expression of chemical defenses in *Alliaria petiolata*. American Journal of Botany. 89: 1422-1430.

Cipollini D, DM Lieurance. 2012. Expression and costs of induced defense traits in *Alliaria petiolata*, a widespread invasive plant. Basic and Applied Ecology, 13: 432-440

Clausen J, DD Keck, W Hiesey. 1940. Experimental studies on the nature of species. I. Effects of varied environments on western North American plants. Carnegie Institution of Washington Publishing. 520

Cocks, PS, JR Phillips. 1979. Evolution of Subterranean clover in South Australia. I. The strains and their distribution. Australian Journal of Agriculture. 30: 1035-1052.

Cousens, R, M Mortimer. 1995. Dynamics of Weed Populations. Cambridge University Press, Cambridge, UK. 335 p

Crooks JA. 2005. Lag times and exotic species: The ecology and management of biological invasions in slow-motion. Ecoscience. 12(3): 316-329.

Daehler CC. 1998. Variation in self-fertility and the reproductive advantage of self-fertility for an invading plant (*Spartina alterniflora*). Evolutionary Ecology. 12: 553-568.

Durka W, O Bossdorf, D Prati, H Auge. 2005. Molecular evidence for multiple introductions of Garlic Mustard (*Alliaria petiolata, Brassicaceae*) to North America. Molecular Ecology. 14: 1697-1706.

Eddmaps, April 2014. University of Georgia Center for Invasive Species and Ecosystem Health. Available from www.eddmaps.org

Ghalambor CK, JK McKay, SP Carroll, DN Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecology. 2: 394–407.

Godoy O, A Saldaña, N Fuentes, F Valladares, E Gianoli. 2011. Forests are not immune to plant invasions: phenotypic plasticity and local adaptation allow *Prunella vulgaris* to colonize a temperate evergreen rainforest. Biological Invasions. 13(7): 1615-1625.

"Eastern U.S." Map. 2014. Google Maps. Google, Web. 13 Apr. 2014.

Grieve M. 1931. A modern herbal; the medicinal, culinary, cosmetic and economic properties, cultivation and folk-lore of herbs, grasses, fungi, shrubs, & trees with all their modern scientific uses. New York: Dover Publications 888 p

Hillstrom C, D Cipollini. 2011. Variation in phenotypic plasticity in native and invasive populations of *Alliaria petiolata*. International Journal of Plant Sciences 172: 763-772.

Hopkins AD. 1920. The Bioclimatic Law. Monthly Weather Review. 48: 355–355.

Karlsson LM, P Milberg. 2008. Variation within species and inter-species comparison of seed dormancy and germination of four annual *Lamium* species. Flora - Morphology, Distribution, Functional Ecology of Plants. 203(5): 409-420

Kinzel W. 1927. Neue Tabellen zu Frost und Licht als beeinflussende Kräfte bei der Samenkeimung.—Stuttgart.

van Kleunen M, D Schlaepfer, M Glaettli, M Fischer. 2011. Preadapted for invasiveness: do species traits or their plastic response to shading differ between invasive and non-invasive plant species in their native range. Journal of Biogeography. 38(7): 1294–1304.

Kowarik I. 1995. Time lags in biological invasions with regard to the success and failure of alien species. In anonymous plant invasions: General aspects and special problems. Amsterdam: Academic publishing. 15-38 p.

Lankau RA, V Nuzzo, G Spyreas, AS Davis. 2009. Evolutionary limits ameliorate the negative impact of an invasive plant. Proceedings of the National Academy of Sciences USA 106:15362–15367.

Lhotská, M. 1975. Notes on the Ecology of Germination of *Alliaria petiolata.* Folia Geobotanica & Phytotaxonomica. 10(2): 179-183.

Martino O, E Mattana, HW Pritchard, G Bacchetta. 2012. Thermal thresholds as predictors of seed dormancy release and germination timing: altitude related risks from climate warming for the wild grapevine *Vitis vinifera* subsp. *sylvestris*. Annals of Botany 110(8): 1651-1660.

Matesanz S, T Horgan-Kobelski and SE Sultan. 2012. Phenotypic plasticity and population differentiation in an ongoing species invasion. Public Library of Science One. 7(9): e44955

Matute, DR. 2013. The role of founder effects on the evolution of reproductive isolation. Journal of Evolutionary Biology. 26 (11): 2299-2311.

Meekins JF, BC McCarthy 2000 Responses of the biennial forest herb *Alliaria petiola*ta to variation in population density, nutrient addition and light availability. Journal of Ecology 88:447–463.

Moloneya KA, C Holzapfelb, K Tielborger, F Jeltschd, FM Schurrd. 2009. Rethinking the common garden in invasion research. Perspectives in Plant Ecology, Evolution and Systematics 11: 311–320

Monty A, G Mahy. 2009. Clinal differentiation during invasion: *Senecio inaequidens* (Asteraceae) along altitudinal gradients in Europe. Oecologia 159:305–315

Monty A, J Lebeau, P Meerts, G Mahy. 2009. An explicit test for the contribution of environmental maternal effects to rapid clinal differentiation in an invasive plant. Journal of Evolutionary Biology 22: 917–926

Mullarkey A, D Byers and R Anderson. 2013. Inbreeding depression and partitioning of genetic load in the invasive biennial *Alliaria petiolata* (*Brassicaceae)*. American Journal of Botany 100(3): 509-518.

Myers CV, RC Anderson. 2003. Seasonal variation in photosynthetic rates influences success of an invasive, exotic plant garlic mustard [*Alliaria petiolata* (M. Bieb.) Cavara and Grande]. American Midland Naturalist. 150(2): 231-245.

NOAA. 2008. NOAA Climate Data: NOAA Observing Systems Architecture. Available from http://www.ncdc.noaa.gov/

Nuzzo V. 1993. Distribution and spread of the invasive biennial *Alliaria petiolata* (garlic mustard) in North America. McNight BN, ed. In Biological Pollution: The Control and impact of invasive exotic species. Indianapolis: Indiana Academy of Science: 134-145.

Nuzzo V. 2000. *Alliaria petiolata* Element Stewardship Abstract. Arlington (VA): The Nature Conservancy.

Parker IM, J Rodriguez, and M Loik. 2003. An evolutionary approach to understanding the biology of invasions: Local adaptation and general-purpose genotypes in the weed *Verbascum thapsus*. Conservation Biology. 17: 59-72.

Prati D, O Bossdorf. 2004. Allelopathic inhibition of germination by *Alliaria petiolata* (*Brassicaceae*). American Journal of Botany. 91:285-288.

Quinn T, MJ Unwin, and MT Kinnison. 2000. Evolution of temporal isolation in the wild: Genetic divergence in timing of migration and breeding by introduced chinook salmon populations. Evolution. 54: 1372-1385.

Raghu A, S Post. 2008. Cold stratification requirements of germination of *Alliaria petiolata*. Invasive Plant Science and Management. 1: 315-318.

Richards CL, O Bossdorf, NZ Muth, J Gurevitch, and M Pigliucci. 2006. Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. Ecology Letters. 9: 981-993.

Rodgers V, K Stinson, and A Finzi. 2008a. Ready or not, garlic mustard is moving in: *Alliaria petiolata* as a member of eastern North American forests. BioScience. 58: 426-437.

Rodgers V, B Wolfe, L Werden, and A Finzi. 2008b. The invasive species *Alliaria petiolata* increases soil nutrient availability in northern hardwood-conifer forests. Oecologia. 157:459-471.

Sakai AK, FA Allendorf, JS. Holt, DM Lodge, J Molofsky, K A With, S Baughman, R J Cabin, J E Cohen, NC Ellstrand, DE McCauley, P O'Neil, I Parker, JN Thompson, and ST Weller. 2001. The population biology of an Invasive Species. Annual Review of Ecology and Systematics. 32:305-332.

Sexton J, J McKay, and A Sla. 2002. Plasticity and genetic diversity of saltcedar to invade cold climates in North America. Ecological Applications. 12:1652-1660.

Sosnoskie LM, and J Cardina. 2009. Laboratory methods for breaking dormancy in garlic mustard (*Alliaria petiolata*) Seeds. Invasive Plant Science and Management. 2009 2: 185–189.

Southeast Climate Consortium. Jan 2013. Chill Accumulation. Available from http://agroclimate.org/tools/Chill-Hours-Calculator/

Stinson K, SA Campbell, JR Powell, BE Wolfe, RM Callaway, GC Thelan, SG Hallett, D Prati, and JN Klironomos. 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting below-ground mutualisms. Public Library of Science Biology. 4: e140-e140.

Susko D, L Lovett-Doust. 1998. Variable patterns of seed maturation in *Alliaria petiolata* (*Brassicaceae*). Canadian Journal of Botany. 70:1677-1686.

Theoharides KA, JS Dukes. 2008. Plant invasion across space and time factors affecting nonindigenous species success during four stages of invasion. New Phytologist. 176:253-273.

Tsutsui NT, AV Suarez, DA Holway, and TJ Case. 2000. Reduced genetic variation and the success of an invasive species. Proceedings of the National Academy of Science. 97: 5948.

USDA plant hardiness zones. 2012. Interactive zone map. Available from http://planthardiness.ars.usda.gov/PHZMWeb/InteractiveMap.aspx

USGS. 2008. Kennesaw Mountain, Cobb County, Georgia. 2008

Vaughn SF, MA Berhow. 1999. Allelochemicals isolated from tissues of the invasive weed garlic mustard (*Alliaria petiolata*). Journal of Chemical Ecology. 25:2495-2504.

Walck JL, SN Hidayati, N Okagami. 2002. Seed germination ecophysiology of the Asian species *Osmorhiza aristata* (*Apiaceae*): comparison with its North American congeners and implications for evolution of types of dormancy. American Journal of Botany. 89(5): 829-35.

Walck J, SN Hidayati. 2004. Germination ecophysiology of the western North American species *Osmorhiza depauperata* (*Apiaceae*): implications of preadaptation and phylogenetic niche conservatism in seed dormancy evolution: Seed Science Research. 14(4): 387-394

Walck J, SN Hidayati, K Dixon, K Thompson, P Poschlod. 2011. Climate change and plant regeneration from seed. Global Change Biology 17: 2145–2161

Wassom JJ, PJ Tranel, M Loyd. 2002. Variation among U.S. accessions of common cocklebur (*Xanthium strumarium*). Weed Technology. 16: 171-179

Welk E, K Schubert, and M Hoffman. 2002. Present and potential distribution of invasive garlic mustard (*Alliaria petiolata*) in North America. Diversity and Distributions. 8:219.

Williams JL, L Fishman. 2014. Genetic evidence for founder effects in the introduced rangeof houndstongue (Cynoglossum officinale). Biological Invasions 16:205–216

Wolfe BE, VL Rodgers, KL Stinson, and A Pringle. 2008. The invasive plant *Alliaria petiolata* (garlic mustard) inhibits ectomycorrhizal fungi in its introduced range. Journal of Ecology. 96: 777-783.